14 $_{31}$Ga Therapeutic Gallium Compounds

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14.1 Introduction

Gallium was described before it was ever observed. This bold feat was accomplished by Dmitri Mendeleev,[1] who in 1871 postulated that 'eka-aluminum' would fill the gap below aluminum in his newly created periodic table of the elements, and would chemically resemble its neighbor. The element was actually discovered in 1875 by Paul-Émile Lecoq de Boisbaudran, not in association with aluminum, but rather as a trace element in sphalerite (zinc sulfide) from southern France. Lecoq de Boisbaudran named the element gallium 'in honor of France (Gallia)',[2] though there have been lingering suspicions that the name also may be derived from 'gallus,' Latin for 'le coq' (rooster).

The first published investigation of gallium's therapeutic potential was that by Levaditi *et al*. in 1931.[3] Gallium tartrate eradicated experimental syphilis in rabbits with a single dose of 30–45 mg Ga/kg intramuscularly or 15 mg Ga/kg intravenously. At 225 mg Ga/kg it was found to eliminate infection by *Trypanosoma evansi* in mice. Despite the reported efficacy and lack of toxicity (750 mg Ga/kg was tolerated in mice), there was no published follow-up to this research over the next several decades.

A period of intensive research on the toxicity and tissue distribution of gallium occurred from about 1949 to 1952. The earliest of these studies showed that

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gallium was poorly absorbed from oral ingestion of common gallium salts or chelates,[4] and that injected gallium (generally as the citrate) tended to concentrate in bone, liver and kidney tissue.[5] Attention quickly turned to gallium radioisotopes, particularly ^{72}Ga, as these could be easily used to trace the distribution of gallium in experimental animals and humans.[6] Radiogallium was soon found to concentrate in regions of high bone turnover, particularly in some bone tumors.[7]

Based on these findings, a preclinical and clinical study (34 patients) was undertaken to explore the use of radioactive ^{72}Ga to treat primary and metastatic bone cancers.[8] The guiding hypothesis was that ^{72}Ga would become highly concentrated in tumors, which would then be destroyed by the localized radiation. Results indicated that due to its short half-life (14.1 h), by the time the ^{72}Ga became concentrated in the target tumors, the radiation level had already decreased so much as to be ineffective, while the patient had been subjected to undesirable levels of whole body radiation.[8] In addition, since ^{72}Ga was produced by neutron irradiation of stable ^{71}Ga, from which it could not be chemically separated, ^{72}Ga was by necessity administered with large amounts of non-radioactive 'carrier' gallium, which was felt to pose a toxicity risk. The use of non-radioactive gallium as a therapeutic agent was apparently not contemplated at this time, and no further work on the potential therapeutic use of gallium appeared until the 1970s. The studies of the early 1950s did, nevertheless, produce data on gallium tissue distribution in experimental animals and humans, and ultimately led to the now widespread use of radioactive gallium (mainly ^{67}Ga, with a half-life of about 78 h) as a diagnostic agent for some cancers, infections and inflammatory diseases.

Research conducted from the early 1970s to the present has demonstrated that non-radioactive gallium has several therapeutic activities, including (a) decreasing accelerated bone mineral resorption and lowering associated elevated plasma calcium levels; (b) inhibiting neoplastic proliferation; and (c) treating certain infectious diseases, particularly those caused by intracellular organisms. In animal models, gallium also has shown selective immunomodulating activity, particularly relating to allograft rejection and autoimmune diseases. Recent reviews[9–11] have covered much of this research and have discussed in detail what is known of gallium's mechanisms of action. Although we are a bit ahead of Mendeleev when he described gallium before it had been observed, we still have only taken a glimpse into gallium's therapeutic potential.

14.2 Chemistry and Mechanisms of Action

14.2.1 Aqueous biochemistry

The chemistry of gallium as it pertains to physiologic solutions has been reviewed,[9,12–14] and will be only briefly summarized here.

Under physiological conditions, gallium is trivalent in aqueous solution (Ga^{3+}).[15] The small and highly charged Ga^{3+} ion (ionic radius 0.62 Å (octahedral) and 0.47 Å (tetrahedral)[16]) is hydrolyzed nearly completely over a wide pH range, forming various hydroxide species, particularly $Ga(OH)_4^-$ (gallate). Concomitant with hydroxide formation in aqueous solutions is the formation of H_3O^+, with resulting lowered pH. If the pH is raised, highly insoluble amorphous $Ga(OH)_3$ precipitates. This $Ga(OH)_3$ tends to convert on aging to the apparently stable crystalline phase GaO(OH).[15] Thus, 'free' Ga^{3+}, which is actually tightly coordinated to six water molecules, has low solubility in most aqueous solutions. At pH 7.4 and 25 °C, overall gallium solubility in equilibrium with crystalline GaO(OH) is approximately 1 μM; there is essentially no unbound Ga^{3+}, and 98.4% of the dissolved gallium is present as $Ga(OH)_4^-$ and 1.6% as $Ga(OH)_3$.[15,17] Both $Ga(OH)_3$ and GaO(OH) display the amphoteric properties first predicted by Mendeleev,[1] becoming increasingly soluble with increasing acidity or basicity, though even at pH 2 their solubility is only approximately 10^{-2} M, and at pH 10 it is only approximately $10^{-3.3}$ M.[15]

These factors of gallium solution chemistry have major implications regarding gallium's therapeutic use. When gallium salts, such as the chloride or nitrate, are dissolved in water (or in other aqueous solutions such as normal saline), most of the gallium ions are hydrolyzed as described above, leaving the resulting solution highly acidic. Over time, such solutions, unless they are extremely dilute or are further acidified, are not stable, and some precipitation of gallium hydroxides will occur. Being acidic, the solutions are not appropriate for parenteral administration by injection. To overcome these problems, gallium solutions for injection are usually prepared with citrate, which chelates the gallium, preventing hydrolysis and improving stability.

Further in this regard, it has been repeatedly observed that low gallium absorption occurs when gallium salts are administered orally.[4,18,19] This low absorption is likely due in large part to the formation of poorly soluble gallium hydroxides in the gastrointestinal tract.

14.2.2 Gallium and iron

The medicinal chemistry of gallium appears to be dominated by the striking similarity in chemical behavior between Ga^{3+} and ferric iron (Fe^{3+}). This similarity, as has been noted,[9] is due to the close correspondence between the two ions in a number of parameters, including ionic radius and factors relating to bond formation (such as electronegativity, ionization potential and electron affinity). Gallium is thus expected to follow many of the same chemical pathways as ferric iron in the body, and to be able to occupy the ferric iron site in some proteins and chelates.

It is the differences between the two ions, however, that allow for gallium's therapeutic potential and that minimize its toxicity. The first major difference is

that Ga^{3+} is essentially irreducible under physiological conditions, whereas Fe^{3+} is readily reducible to Fe^{2+} (a much more soluble and significantly larger ion). This difference means that, *in vivo*, Ga^{3+} does not enter Fe^{2+}-bearing molecules such as heme (a fundamental component of hemoglobin and myoglobin, as well as cytochromes and numerous other enzymes) and thus does not interfere with oxygen transport and other vital functions. It also means that when Ga^{3+} substitutes for Fe^{3+} in a redox-active enzyme, it cannot participate in redox reactions, making the enzyme non-functional. Furthermore, Ga^{3+} will not participate in Fenton-type redox reactions, in which hydroxyl and other highly reactive oxygen-bearing free radicals are produced; such reactions make unbound iron ions highly toxic when present in blood plasma. The other major difference is that Fe^{3+} is even less soluble in neutral aqueous solutions than is Ga^{3+}: at pH 7.4 and 25 °C, the solubility of Fe^{3+} (in equilibrium with FeO(OH)) is only approximately 10^{-18} M, compared to the analogous figure of approximately 10^{-6} M for Ga^{3+}.[14] This difference means that whereas essentially all Fe^{3+} is protein-bound or chelated in blood plasma, small but potentially significant amounts of gallate ($Ga(OH)_4^-$) can exist at equilibrium, which can participate in activities not available to the protein-bound metal.

Because unbound iron ions are highly toxic, iron is maintained bound to a series of proteins and small molecules when it is absorbed and transported throughout the body.[20] In blood plasma, iron exists predominately as Fe^{3+} bound to transferrin (TF), which is the major iron transport protein. Similarly, at equilibrium, nearly all Ga^{3+} in blood plasma is bound to TF.[9,13] Metal-bearing TF, particularly when saturated with two metal ions, can bind to transferrin receptor (TFR) on cell membranes; this complex is taken up by endocytosis, the metal is released from TF as the endosome is acidified to pH 5.5, and the TF and TFR are recycled.[20] TFR is expressed in all nucleated cells, but at the highest levels in many neoplastic cells, as well as in normal hepatocytes; Kupffer cells; erythroid precursors; and cells of the basal epidermis, endocrine pancreas, seminiferous tubules and mucosal epithelium.[21–23] These cells all have a high need for iron: rapidly proliferating cells, particularly neoplastic cells, must manufacture the Fe^{3+}-bearing enzyme ribonucleotide reductase, which is essential for DNA synthesis; erythroid precursors (mainly in the marrow) reduce Fe^{3+} to Fe^{2+} to produce hemoglobin and related molecules; and liver cells as well as macrophages absorb and store iron to regulate overall iron levels.

The iron-binding capacity of TF in blood plasma is normally approximately 3.3 μg of Fe^{3+}/ml (referred to as the total iron-binding capacity), though normally only about 33% of the available metal-binding sites (two per TF molecule) are occupied by Fe^{3+}.[24] Thus, at normal iron saturation levels, plasma TF has the capacity to bind as much as approximately 2.7 μg/ml (40 μM) of Ga^{3+}; if Ga^{3+} concentrations exceed this level, then significant amounts of gallate will form, together with traces of $Ga(OH)_3$ and gallium citrate.[9]

Gallium also binds to the Fe^{3+} sites of lactoferrin, a protein closely related structurally to transferrin. Lactoferrin binds Fe^{3+} and Ga^{3+} more avidly than does TF, and can remove Ga^{3+} from TF.[25] Apolactoferrin exerts anti-microbial activity by locally sequestering iron, an essential nutrient, and occurs in amounts of approximately 0.5–1 mg/ml in epithelial secretions such as milk, tears, seminal fluid and nasal discharge.[26,27] It is also secreted at sites of infection and inflammation.[28–30]

Ferritin, a very large (440 kDa) protein used for iron storage, will also bind gallium. Gallium can be transferred to ferritin from transferrin or lactoferrin, with ATP and other phosphate-bearing compounds acting as mediators.[31,32] Ferritin is present in most cell types, but is concentrated in Kupffer cells of the liver and other tissue macrophages, and in duodenal mucosal cells, particularly when there is abundant iron in the diet.[20,24]

The binding of gallium to transferrin, lactoferrin and ferritin accounts for much of the tissue distribution observed when gallium radioisotopes are administered. ^{67}Ga is found to concentrate most highly in many tumors and at sites of inflammation and infection.[33–35] Many tumors overexpress TFR, and the avidity of tumors for ^{67}Ga has been correlated to transferrin receptor 1 (TFR1; CD71) expression.[14,36–39] Exceptions to this generalization exist however, and gallium appears to enter some ^{67}Ga-avid tumors by TFR1-independent mechanisms.[40–42] A second membrane-bound transferrin receptor (TFR2α) has been described,[43] which is most highly expressed in hepatocytes, some erythroid cells and the crypt cells of the duodenum (which sense plasma iron levels and then, when they mature into villi enterocytes, regulate dietary iron absorption).[43–45] Some of the reported non-transferrin-receptor-mediated gallium uptake is likely through TFR2α. Little information is available on the direct uptake by cells of gallium bound to low molecular weight (LMW) molecules; it is noted, however, that Ga^{3+}, as well as Fe^{3+} and other trivalent or tetravalent metal ions, greatly upregulates the uptake of LWM-bound Fe by monocytes, macrophages, neutrophils and myeloid cells, as well as the binding of transferrin and lactoferrin to cell membranes.[46] The mechanism of LMW-bound ferric iron absorption may apply to LMW-bound gallium.

The concentration of ^{67}Ga at sites of inflammation and infection likely stems from its binding to lactoferrin, as well as from uptake by some leukocytes[35,47,48] and, when present, bacteria.[49,50]

Although gallium is avidly taken up by a wide range of proliferating cancer cells, it is not concentrated by rapidly proliferating normal cells, such as gastrointestinal mucosal cells, hematopoietic cells of the bone marrow and elsewhere, and the transient cells of hair follicles; significant ^{67}Ga accumulations do not occur at these sites. The reasons for the lack of accumulation in normal proliferating tissue have not been explored, but are likely due in part to local recycling of iron, so that little new uptake of iron (or gallium) from plasma occurs.

14.2.3 Mechanisms of action

The mechanisms for the observed therapeutic activities of gallium have been reviewed,[9] so will be only briefly summarized here.

Much of gallium's therapeutic activity derives from its ability to mimic Fe^{3+} and yet not to participate in the redox reactions available for Fe^{3+}. This mimicry leads to the concentration of gallium at sites in the body where Fe^{3+} is taken up from plasma, including proliferating cancer cells; infected cells, particularly macrophages; and proliferating bacteria and parasites. When gallium reaches these sites it will compete with Fe^{3+} and will interfere with its absorption, metabolism and activity.

Iron is essential to cell division, largely because it is present in the active site of ribonucleotide reductase, an enzyme that catalyzes the production of the deoxyribonucleotides required for DNA. By competing with iron, gallium can interfere with ribonucleotide reductase activity and inhibit DNA synthesis.[51] Furthermore, Ga^{3+} can substitute for Fe^{3+} in the M_2 subunit of ribonucleotide reductase, deactivating the enzyme through a conformational change.[52,53] If DNA synthesis is inhibited in proliferating cells, apoptosis may result: in human leukemic CCRF-CEM cells deprived of iron, by exposure to either deferoxamine or 12.5–100 μM gallium nitrate, apoptosis is induced.[54] Similarly, exposure of human peripheral blood mononuclear cells to 50–100 μM gallium nitrate induces apoptosis.[55]

Cancer cells that take up gallium become iron-deprived, which causes upregulation of TFR1.[56] Increased TFR1 promotes increased Ga-TF uptake, leading to increased iron deprivation, and this cycle continues until apoptosis of the cell results. Ga-TF may cause additional iron deprivation by preventing sufficient acidification of Fe-TF-containing endosomes to allow for the intracellular release of Fe.[56]

Several *in vitro* studies[57,58] have found that Ga^{3+} can directly bind to DNA, and may compete with magnesium in this regard. As these studies were done using isolated DNA at pH values of 4–5, their relevance to *in vivo* gallium activity is not clear.

Gallium nitrate and Ga-TF were found to potently inhibit protein tyrosine phosphatase (PTPase) from Jurkat human T-cell leukemia cells and HT-29 human colon cancer cells (IC_{50} value of 2–6 μM).[59] This activity did not, however, correlate with growth inhibition in these cells, and a relationship between the observed PTPase inhibition and gallium's anti-tumor activity has not been established.

As mentioned, gallium inhibits the proliferation of some pathogenic microorganisms. It may be particularly effective in treating some intracellular pathogens, such as species of *Mycobacterium*.[60] Infected cells (particularly macrophages) take up Ga-TF; the infecting organisms then take up gallium instead of iron and, as with cancer cells, cannot synthesize sufficient DNA for replication, and ultimately die.

In addition to these anti-proliferative activities, gallium is potently anti-resorptive to bone mineral, appears to have anabolic (formation-stimulating) effects on bone, inhibits some T-cell and macrophage activation and has other selective immunomodulatory activities.[9]

14.3 Therapeutic Gallium Compounds

At present, only citrated gallium nitrate (Ganite™) is approved for therapeutic use (in the United States, for cancer-related hypercalcemia). Based on publicly available information, the only other potentially therapeutic gallium compounds that have been in clinical trials are gallium chloride, gallium maltolate and gallium 8-quinolinolate. Gallium-transferrin and a gallium–transferrin–doxorubicin conjugate have been administered to a small number of cancer patients, and several other gallium compounds have undergone preclinical testing.

14.3.1 Gallium nitrate and citrated gallium nitrate

Most data on the biological and therapeutic activities of gallium derive from investigations using gallium nitrate in aqueous solution. The term 'gallium nitrate,' however, has not been used consistently: it has been used to describe (a) chelator-free gallium nitrate solutions, employed for most of the *in vitro* and some of the animal studies and (b) gallium nitrate solutions containing citrate as a chelator, employed for all of the clinical studies and some of the animal studies. Chelator-free gallium nitrate solutions contain ionic gallium (mostly as the pH-dependent hydroxide species discussed previously), whereas citrate-containing solutions at neutral pH contain gallium citrate as a coordination complex. The commercially available product for injection (Ganite™) contains 97.8 mM of both gallium and citrate at neutral pH,[61] which allows essentially all the gallium to bind to citrate, with very little gallium remaining in other forms. This solution is here referred to as 'citrated gallium nitrate' or 'CGN,' whereas gallium nitrate without citrate or other chelators is abbreviated 'GN.' Dose levels for both GN and CGN are given in terms of anhydrous gallium nitrate ($Ga(NO_3)_3$).

In vitro *and non-human* in vivo *studies*

Anti-tumor activity of GN was first reported in 1971:[62] efficacy was demonstrated against Walker 256 ascites carcinosarcoma in rats ($GaCl_3$ and $Ga(SO_4)_3 \cdot 18H_2O$ had very similar effects), and >90% growth inhibition was

observed for six of eight solid tumors implanted subcutaneously (sc) in rodents. Complete tumor regression occurred in several animals. Unchelated, acidic GN solutions were administered intraperitoneally (ip) daily for 10 days; LD_{10} (at 30 days) was 63 mg/kg (mouse), 50 mg/kg (rat); LD_{50} was 80 mg/kg (mouse), 67.5 mg/kg (rat); and the effective dose varied from 30 to 60 mg/kg. *In vitro*, GN gave an ID_{50} of 355 μM (anhydrous basis) against Walker 256 carcinosarcoma cells.[63] *In vivo* efficacy against implanted human medulloblastoma (Daoy cells) was observed in nude mice that received ip GN (50 mg/kg/day for 10 or 15 days): the growth rate of macroscopic tumors was reduced or reversed[64] and the progression of microscopic disease delayed.[65]

The finding of an anti-hypercalcemic effect in humans, which results from inhibited bone resorption rather than increased urinary calcium excretion,[66,67] stimulated considerable research on gallium's bone-related activity (reviews: [9,68]). Gallium concentrates in skeletal tissue, particularly at sites of rapid bone mineral deposition such as active metaphyseal growth plates and healing fractures.[5,6,69,70] Concentration on the endosteal and periosteal surfaces of diaphyseal bone also occurs, but to a lesser extent.[70] Several studies (using bone fragments *in vitro* or implanted in rodents) found that gallium adsorbs to bone surfaces and then inhibits osteoclastic bone resorption;[71–73] gallium-treated bone is also less soluble in acetate buffer and less readily resorbed by monocytes.[74] Gallium does not enter the crystal lattice of hydroxylapatite, but rather deposits in part on bone mineral surfaces, possibly as gallium phosphate.[75] At anti-resorptive concentrations (up to at least 15 μM, and possibly >100 μM), GN does not affect osteoclast morphology or viability,[67,71,73] or act as an osteoclastic metabolic inhibitor, as do bisphosphonates.[76] Gallium is found to directly inhibit the osteoclast vacuolar-class ATPase proton pump.[76] In young female rats, administration of ip GN resulted in bone containing more calcium, having a larger average hydroxylapatite crystallite size, and having a higher density relative to that in untreated individuals.[69,74]

Some *in vitro* data indicate that GN may stimulate bone formation. Experiments with rat osteogenic sarcoma (ROS) and normal rat osteoblast cells found that GN can decrease osteocalcin (OC) and OC mRNA levels[77,78] and increase c-fos mRNA levels;[79] both activities are associated with bone formation. *In vivo* and clinical studies have not shown clear, consistent anabolic activity, though elevated plasma alkaline phosphatase (a marker of bone formation) was observed in post-menopausal women treated with CGN.[80]

Gallium nitrate and other gallium salts have shown selective *in vitro* and *in vivo* immunomodulating activity. This activity appears to stem primarily from inhibition of some T-cell activation and proliferation[48,80–82] and inhibition of inflammatory cytokine secretion by activated macrophages,[80,83,84] without generalized cytotoxicity to lymphocytes or macrophages. Some of the selective anti-inflammatory activity may be due to pro-inflammatory T-helper type 1 (Th-1) cells being much more sensitive to inactivation by iron deprivation than anti-inflammatory, pro-antibody Th-2 cells.[85] Synoviocyte matrix

metalloproteinase activity (elevated in inflammatory joint diseases) was also shown to be dose-dependently inhibited by GN at concentrations of 10–100 μM.[86] In animal models, GN has shown efficacy in suppressing adjuvant-induced arthritis,[80] experimental encephalomyelitis,[81] experimental autoimmune uveitis,[87] type 1 diabetes,[88] endotoxic shock,[89,90] and allograft rejection.[91]

Clinical experience

Parenterally administered CGN has demonstrated single-agent clinical efficacy in cancer-related hypercalcemia (for which it is approved in the United States), Paget's disease of bone, and several types of cancer, including lymphoma (43% response in relapsed malignant non-Hodgkin's lymphoma),[66,92] advanced refractory urothelial carcinoma (17% response),[93] advanced bladder carcinoma (40% average response in two studies),[94,95] advanced or recurrent epithelial ovarian carcinoma resistant to cisplatin (12% response),[96] metastatic or advanced non-squamous cell cervical carcinoma (12% response),[97] advanced or recurrent squamous cell carcinoma (8% response)[98] and metastatic prostate cancer (2 of 23 patients had partial response, and 7 had reduction in bone pain after treatment for only 7 days).[99]

Early anti-cancer clinical trials employed short (generally $\leq$30 min) iv bolus infusions at doses up to 1350 mg/m^2;[100] subsequently, only prolonged (generally $\geq$5 days) iv infusions or sc injections have been used, due to dose-limiting renal toxicity from bolus iv dosing.

For cancer-related hypercalcemia, CGN is administered as a continuous iv infusion for 5 days at 200 mg/m^2/day ($\sim$5 mg/kg/day).[101,102] Studies comparing CGN to other anti-hypercalcemic drugs found that the proportion of patients achieving normocalcemia was higher for those receiving CGN than for those receiving pamidronate,[103] calcitonin[101] or etidronate.[104]

A small study[105,106] found that CGN may be particularly efficacious against multiple myeloma. Thirteen relapsed multiple myeloma patients (11 at stage III) were treated for 6 or 12 months with sc CGN at 30 mg/m^2/day, administered for two weeks followed by two weeks without therapy, together with the M-2 chemotherapy protocol. A 5-day iv infusion of 100 mg/m^2/day CGN was administered every other month. These patients were matched with 167 similar patients who received only M-2 therapy. Patients receiving CGN showed an increase in total body calcium (decreased bone resorption), stabilized measures of bone density, reduction in vertebral fractures and decreases in measures of pain versus those not receiving CGN. Significantly, mean survival in the CGN-treated group was 87+ months, with several long-term survivors (including stage III patients (at presentation) alive at 137+ and 144+ months, and a stage IIIB patient alive at 96+ months in complete remission), compared to mean survival of 48 months in the 167 patients who received M-2 chemotherapy alone, with no long-term survivors.

Markers of bone turnover were significantly reduced in drug-resistant patients with Paget's disease of bone (a disease characterized by localized, greatly accelerated bone remodeling) who received relatively low doses of CGN (iv at 100 mg/m^2/day for 5 days[107] or sc at 0.25 or 0.5 mg/kg/day in two non-consecutive 14-day cycles).[108] No drug-related adverse events were reported other than moderate, transient reductions in hemoglobin and serum iron-binding capacity.

Pharmacokinetics

Data on the pharmacokinetics of CGN are sparse and show significant individual variability;[109–112] much of the variability may be due to differences in renal function or the extent of metastatic disease (because neoplastic tissue and associated areas of inflammation may take up significant amounts of gallium). Steady-state plasma gallium levels of 1.2–1.5 μg/ml were achieved within 48 h in eight hypercalcemic patients who received 200 mg/m^2 CGN as a continuous infusion for 5 days, and levels of 1.0–1.3 μg/ml were achieved in six patients who received 100 mg/m^2 on the same schedule (plus one patient whose plasma gallium level never exceeded 0.45 μg/ml).[112] A gallium plasma level of 1 μg/ml was considered therapeutic for cancer-related hypercalcemia in this study. The non-linear dose/plasma concentration relationship was not discussed. Elimination of gallium following administration of iv CGN is considered biphasic, with an initial elimination half-life of 0.15 to 1.5 h and a terminal half-life of 6 to 196 h; more than half of the administered dose is generally excreted in the urine within 24 h.[109–111] In a single patient who received CGN at 0.5 mg/kg (20 mg/m^2) sc daily for 14 days, maximal gallium plasma concentrations of 0.95 to 1.85 μg/ml occurred 1–2 h after injection; 19% of the administered gallium was renally excreted on day 1, and 28% by day 7.[113]

Toxicity

The major toxicity associated with iv CGN is renal, as indicated by proteinuria, increases in blood urea nitrogen (BUN) and serum creatinine, and a decrease in creatinine clearance. Rats given ip CGN at 100 mg/kg develop renal tubule damage, which is caused at least in part by precipitation of gallium and calcium phosphates within the tubule lumina.[114] In humans, renal toxicity is consistently observed following a 30-min infusion of $\geq$750 mg/m^2, though it is ameliorated by concomitant mannitol diuresis.[110] At lower doses or with longer infusion times, renal toxicity is reduced: in well-hydrated patients receiving 300–400 mg/m^2/day for 7 days, markers of renal tubule damage are not significantly elevated,[115] and well-hydrated hypercalcemia patients receiving 200 mg/m^2/day for 5 days rarely show significant impairment of creatinine clearance.[101,104,112]

Another dose-related toxicity observed is anemia, which is generally mild (<3 g/dl decrease in hemoglobin) and has only been clinically significant at high bolus iv doses.[100,110] When CGN is administered as prolonged infusions at 200–300 mg/m^2, decreases in hemoglobin are usually no more than 1–2 g/dl.[66,105,108] The anemia, when observed, is similar to that arising from iron deficiency, with hypochromia and microcytosis; iron-binding capacity is slightly decreased, while serum iron, TF and ferritin levels are normal.[116]

Other adverse events occasionally observed following slow iv infusion of CGN that may be drug-related include nausea and vomiting,[96] mild respiratory alkalosis,[116] hypophosphatemia,[116] hypocalcemia [116] and optic neuritis.[117]

14.3.2 Gallium chloride

Gallium chloride ($GaCl_3$; GC) has shown anti-cancer activity in animal studies; activity was greatest during the exponential growth phase of tumors.[118] Animal studies found strong preferential uptake by tumor cells when GC was administered orally,[119] without the high renal uptake and toxicity observed following ip administration.[120]

Oral GC was administered to 18 lung cancer patients for 15 days at doses ranging from 100 to 1400 mg/day.[18] Bioavailability was low and serum gallium levels did not increase at doses over 400 mg/day. After 15 days, a steady-state gallium serum level of 371 ± 142 ng/ml was reached; the level was significantly lower in patients with metastases than in those without. Mild reductions in hemoglobin and magnesium were observed.[121] The maximum accessible serum concentrations were deemed too low for GC to be used as a single agent in lung cancer.[18] Possible potentiation of cisplatin and etoposide therapy by GC in lung cancer patients was reported.[122]

14.3.3 Gallium 8-quinolinolate

Gallium 8-quinolinolate (tris(8-quinolinolato)gallium(III); GQ; Figure 14.1) was developed to provide high oral gallium bioavailability for use in cancer treatment.[123] GQ inhibited the *in vitro* proliferation of A549 human lung adenocarcinoma cells: the IC_{50} was 2.5 μM, similar to that for GN and about a tenth the value for GC.[124] In rats transplanted with Walker carcinosarcoma 256, tumor reduction of >50% was observed following oral gavage at 24 mg/kg/day on days 3–9 after transplantation; an equimolar oral dose of GN had no anti-tumor effect.[125] An anti-hypercalcemic effect was also observed. A dose of 62.5 mg/kg/day for two weeks was well tolerated in healthy Swiss mice, whereas doses of 125 mg/kg/day proved toxic (including leucopenia, but not anemia) and sometimes fatal.[123] In the animals that received 62.5 mg/kg/day, gallium was concentrated in the bone (7(3) μg/g), liver (4(2) μg/g), spleen (2(1) μg/g) and

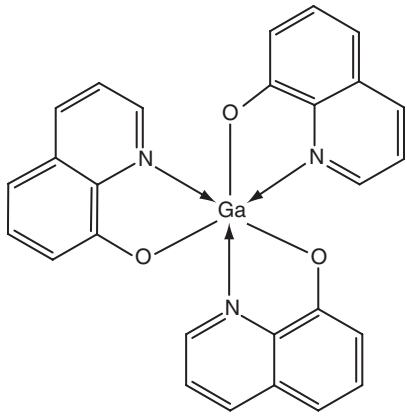

Figure 14.1 Structure of tris(8-quinolinolato)gallium(III) (gallium 8-quinolinolate; GQ)

kidneys (1.8(2) μg/g), with <0.03 ng/g gallium found in the brain, lungs, testes, and ovaries (serum concentration not given).[123] This compound entered human clinical trials in 2004 (B. Keppler, personal communication, 2004).

14.3.4 Gallium maltolate

Gallium maltolate (tris(3-hydroxy-2-methyl-4*H*-pyran-4-onato)gallium(III); GM; Figure 14.2) is an orally active complex that is moderately soluble in both aqueous and lipidic solutions (octanol/water partition coefficient of 0.41(8)) and is stable over a pH range of about 5.5–8.0.[126] The compound has shown oral gallium bioavailability of at least 25–57% in healthy human subjects, with roughly linear absorption and elimination kinetics following single doses of 100–500 mg and an average maximum serum gallium level (C_{max}) of 569(17) ng/ml at the 500-mg dose.[126] Similar results were obtained in elderly subjects with Paget's disease of bone; in these patients it was found that C_{max} was 1138(532) ng/kg after 3 days of dosing at 600 mg/day.[127] Only about 2% of administered gallium was excreted in the urine 72 h after dosing, and no renal toxicity was observed.[126] These results strongly suggest that plasma gallium following oral GM administration is nearly all bound to transferrin; in contrast, much of the plasma gallium following iv CGN administration is in the form of gallate, which is rapidly excreted in the urine and may cause renal toxicity.[126] The drug appears well tolerated at doses of up to 600 mg/day for 3 days.[126,127]

In animal studies, oral GM has shown efficacy in some rat models of rheumatoid arthritis, in which inflammation and bone degradation were

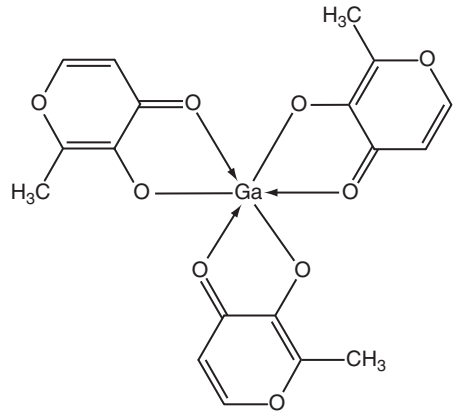

Figure 14.2 Structure of tris(3-hydroxy-2-methyl-4*H*-pyran-4-onato)gallium(III) (gallium maltolate; GM)

suppressed (A. Bendele, personal communication, 2004); tuberculosis in guinea pigs;[128] and *Rhodococcus equi* infection in mice.[129]

14.3.5 Other gallium compounds

A gallium–doxorubicin–transferrin conjugate was 100 times more inhibitory to a doxorubicin-resistant MCF-7 human breast cancer cell line than doxorubicin alone, and 10 times more inhibitory than an iron–doxorubicin–transferrin conjugate.[130] Ga-TF in conjunction with doxorubicin and other chemotherapeutic agents, sometimes with a preliminary dose of deferoxamine, has shown anti-tumor activity and low toxicity following iv administration to breast cancer patients (J. Head, personal communication, 2004).

Gallium chelated to pyridoxal isonicotinoyl hydrazone (PIH; an iron chelator that can enter cells through a non-TFR-mediated mechanism) (Figure 14.3) was found to be growth inhibitory in a human T-lymphoblastic leukemic CCRF-CEM cells resistant to gallium alone, and was more inhibitory than PIH alone.[131]

A complex of gallium with 2-acetylpyridine ^4N-dimethylthiosemicarbazone and chloride (Figure 14.4) showed IC_{50} values in the low nanomolar range against ovarian (41M), mammary (SK-BR-3) and colon (SW480) carcinoma cells, though the values were similar to those of the ligand alone.[132] Further testing is needed to determine whether the gallium helps target the compound to gallium-avid cancer cells.

Gallium protoporphyrin IX (GaPPIX; Figure 14.5) at concentrations of about 1 μg/ml showed *in vitro* growth-inhibitory activity against many pathogenic

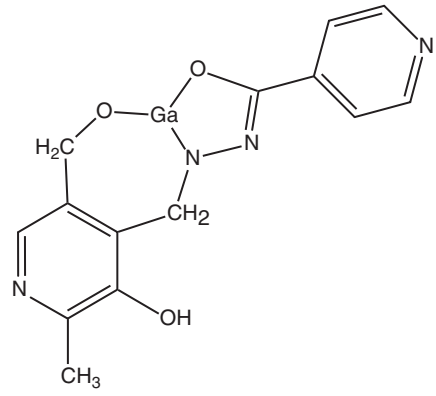

Figure 14.3 Structure of pyridoxal isonicotinoyl hydrazone gallium(III)

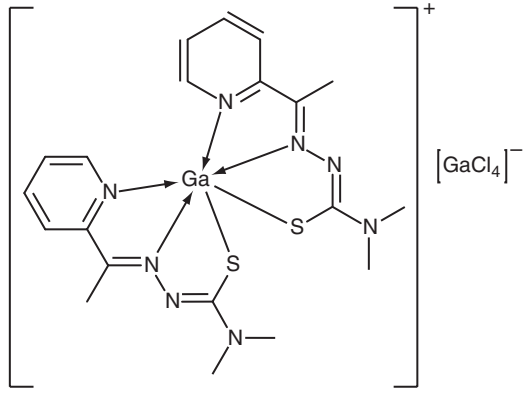

Figure 14.4 Structure of bis(2-acetylpyridine ^{4}N-dimethylthiosemicarbazone)gallium(III), gallium(III) tetrachloride

bacteria, including Gram-positive and -negative species and mycobacteria,[133] and against the malarial parasite *Plasmodium falciparum*.[134] Protoporphyrin IX or gallium alone had approximately one-hundredth the anti-bacterial activity of intact GaPPIX against the most sensitive organisms.[133] GaPPIX appears to enter bacteria primarily through heme uptake systems; toxicity may occur by incorporation of the molecule into cytochrome/quinol oxidases, which leads to the generation of reactive oxidative species in locally cytotoxic concentrations.[133] Human primary fibroblasts exposed to 100 μg/ml GaPPIX for 48 h displayed no decrease in viability, and mice given 25–30 mg/kg ip, followed by four daily ip doses of 10–12 mg/kg, showed no acute toxic effects.[133]

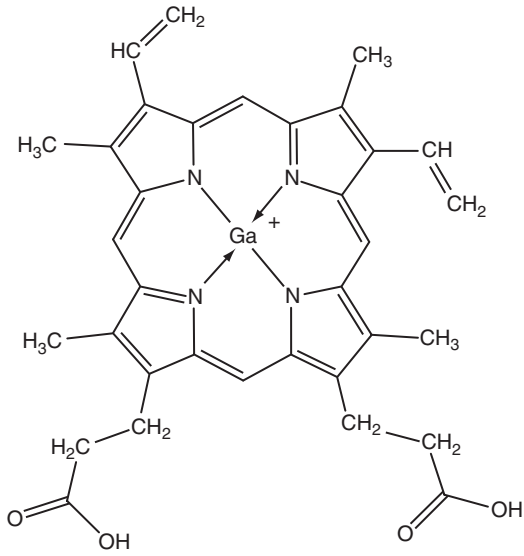

Figure 14.5 Structure of gallium(III) protoporphyrin IX (GaPPIX)

Abbreviations Used

ATP, adenosine triphosphate; CGN, citrated gallium(III) nitrate aqueous solution; GaPPIX, gallium(III) protoporphyrin IX; GC, gallium(III) chloride; GM, gallium(III) maltolate; GN, gallium(III) nitrate; GQ, gallium(III) 8-quinolinolate; ip, intraperitoneal; iv, intravenous; PIH, pyridoxal isonicotinoyl hydrazone; PTPase, protein tyrosine phosphatase; sc, subcutaneous; TF, transferrin; TFR, transferrin receptor.

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